TWO CIS-PROLINES IN THE BENCE-JONES PROTEIN REI AND THE CIS-PRO-BEND

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1. Introduction

The stabilities of the cis- and trans- forms of the X-Pro peptide group differ only slightly by −2.0 to 2.0 kcal/mol^{-1} for linear polypeptides [1]. In small cyclic peptides, the ring closure enforces the formation of cis-peptide groups [2,3]. It is surprising, therefore, that there are only few reports of X-Pro cis-peptide groups found in globular protein molecules. Furthermore, most of these are questioned by the authors due to the uncertainty in the interpretation of a Fourier map calculated with phases obtained from isomorphous replacement [4,5a,5b,6–8]. During the course of the constrained crystallographic refinement of the crystal structure of the Bence-Jones Protein Rei [9a,9b] we found evidence for two X-Pro cis-peptide groups and were able to confirm this by difference Fourier methods.

2. Crystallographic refinement

The constrained crystallographic refinement used had already been applied to the crystal structures of PTI [10a,10b] and PTI–trypsin complex [11a,11b]. It has been shown that those protein structures had been refined to a final accuracy of better than 0.1 Å and 0.2 Å respectively using this procedure.

The refinement procedure consists of cycles of phase calculation, Fourier-synthesis and real space refinement [12]. Difference Fourier-syntheses are inserted and analysed to detect gross errors of the model which the automated procedure cannot correct. A refinement was performed with the Rei crystal structure using intensity data to 2.2 Å resolution [9b]. After several rounds of refinement cycles and difference maps, we found large density residuals at the Ser 7-Pro 8 and Leu 94-Pro 95 segments which had been built in the trans-conformation. The local distribution of maxima and minima around these two peptide groups was very similar and consistent in the two independent molecules present in the asymmetric unit. Fig. 1a shows 6 consecutive sections through the difference Fourier map, calculated with a Ser 7-Pro 8 trans-peptide group. There is a large negative density at the positions of the Ser 7 carbonyl carbon and oxygen atoms and there is high positive density unaccounted for at sections 5 and 6. This clearly suggested the presence of a cis-peptide group, which would have its carbonyl group approximately in the position of C^{β} of Ser 7 in the trans-peptide. C^{β}–O^{γ} of Ser 7 would occupy the residual positive density. A model was then constructed with Ser 7-Pro 8 and Leu 94-Pro 95 cis-peptide groups and refined as described above.

The finally calculated difference Fourier map is shown in fig. 1b. It is practically featureless. The positive density visible in the lower right hand corner of sections 5 and 6 is due to the side-chain of Gln 24, which is misplaced. The refinement has progressed to a crystallographic R-value of 0.26.

$$R = \frac{\Sigma \| F_o | - | F_c \|}{\Sigma | F_o |},$$

summation over all 13 000 reflections. F_o–observed

The nomenclature recommended by IUPAC-IUB [14] is used in this paper.

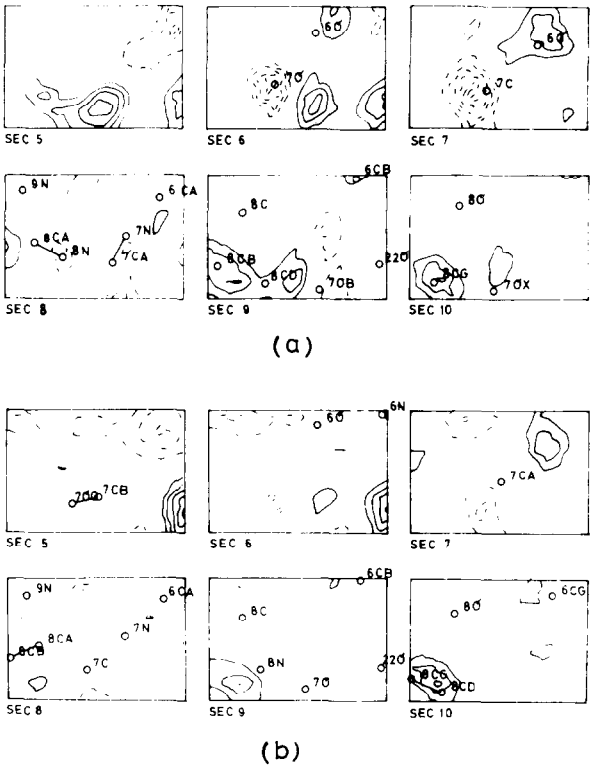

Fig. 1. a) Difference Fourier map at the segment around Pro 8 calculated with a Ser-Pro trans-peptide group. Contour levels at ± 0.05 e/Å^3, starting at ± 0.1 e/Å^3. Negative contour levels dashed. 70X is O_7^γ, which has been excluded from the calculations. b) Difference Fourier map at the segment around Pro 8 calculated with a Ser-Pro cis-peptide group. Contour levels as above.

Table 1
Main chain torsion angles in the two cis-Pro-bends in V_{Rei}

	ϕ	degrees	ψ
Gln 6	−106		138
Ser 7	−145		139
Pro 8	− 62		174
Ser 9	− 95		−18
Ser 93	−137		155
Leu 94	− 82		150
Pro 95	− 73		156
Tyr 96	− 71		125

The angles are averages over the two molecules in the asymmetric unit of the crystal cell.

structure factor amplitude, F_c—calculated structure factor amplitude. The difference map shows a few regions where the model is still in error, but these are far from the two proline residues discussed and further refinement will not influence these segments.

Table 1 shows the main chain torsion angles of the residues around Pro 8 and Pro 95, averaged over the two independent molecules. The two independent chains are very similar, the average deviation being 8° in torsion angles. Fig. 2 is a stereo-plot of the electron density and its model interpretation in the vicinity of Pro 8 showing a very satisfactory fit.

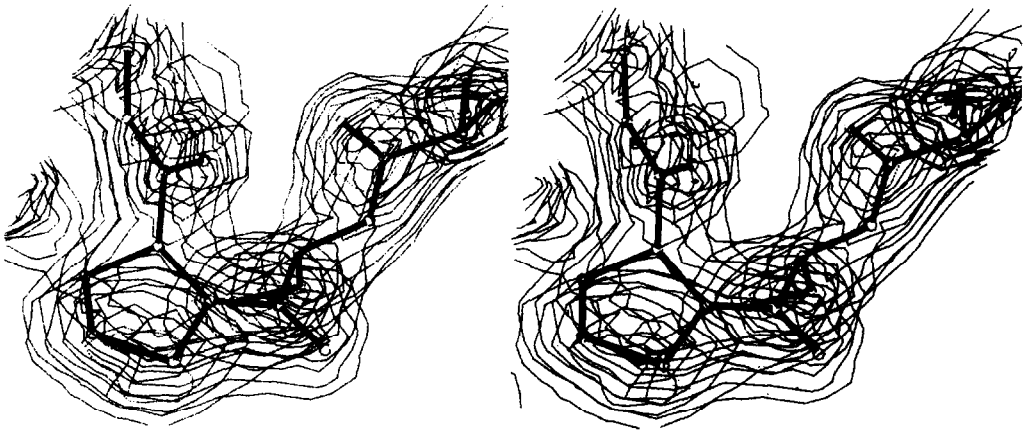

Fig. 2. Electron density map and corresponding model interpretation at Pro 8. Contour levels in steps of 0.2 e/Å^3, starting from 0.2 e/Å^3. Ser 7 is at the lower right hand side. Its C^β–O^γ lies below the Ser-Pro peptide group.

3. Discussion

The demonstration of two cis-prolines leads to a general consideration of the underlying structural principles. Both residues 8 and 95 lie in the third position of reverse turns which may be characterized as open turns according to the definitions suggested in ref. [13]. The C_1^{α}–C_4^{α} (first and fourth residue in the turn) distances are less than 6.0 Å, but there is no hydrogen bond between O_1 and N_4.

In trying to build a reverse turn using conventional model parts with proline at the third position, unallowed close contacts between C_2^{β} and C_3^{δ} of proline are observed. Overcrowding between C_3^{δ} and C_2^{β} or N_2 occurs over a considerable range of the ψ_2 torsion angle. A glycine in the second position should of course make a trans-proline possible. Building the reverse turn with a X-Pro cis-peptide group, C_2^{β} is removed far from C_3^{δ} of the proline residue (see fig. 2). An inspection of stereo-drawings and atomic coordinates of other globular protein molecules, where cis-prolines have been reported, showed that in all of these cases the proline residues are at the third position of a reverse open turn: ribonuclease (Pro 93 and 114) [7], thermolysin (Pro 51) [4], sustilisin (Pro 168) [5a, 5b], erythrocruorin (Pro EF3, 74) [6], and carbonic anhydrase (Pro 29 and 200) [8].

There appears to be a considerable variation in the main chain conformational angles of these various Cis-Pro-bends (table 1). None of these (except perhaps in subtilisin) shows O_1–N_4 hydrogen-bonding. There is no indication of a certain aminoacid sequence preferring this conformation, possibly due to the small number of observations available as yet. The observation of 9 cis-Pro-bends in 6 different protein molecules suggests that this is an important structural element.

In view of the uncertainty of protein models obtained from Fourier maps calculated with isomorphous phases, a reexamination of segments around proline residues in other protein structures might be advisable.

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